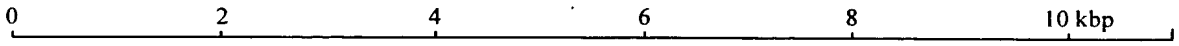
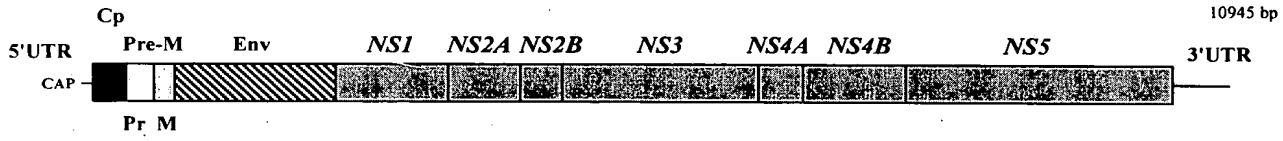


Genomic organization of WNV-HNY1999



Cloning Strategy for WNV-HNY1999 Capsid Gene: pWNVh-DJY, pWNVy-DJY

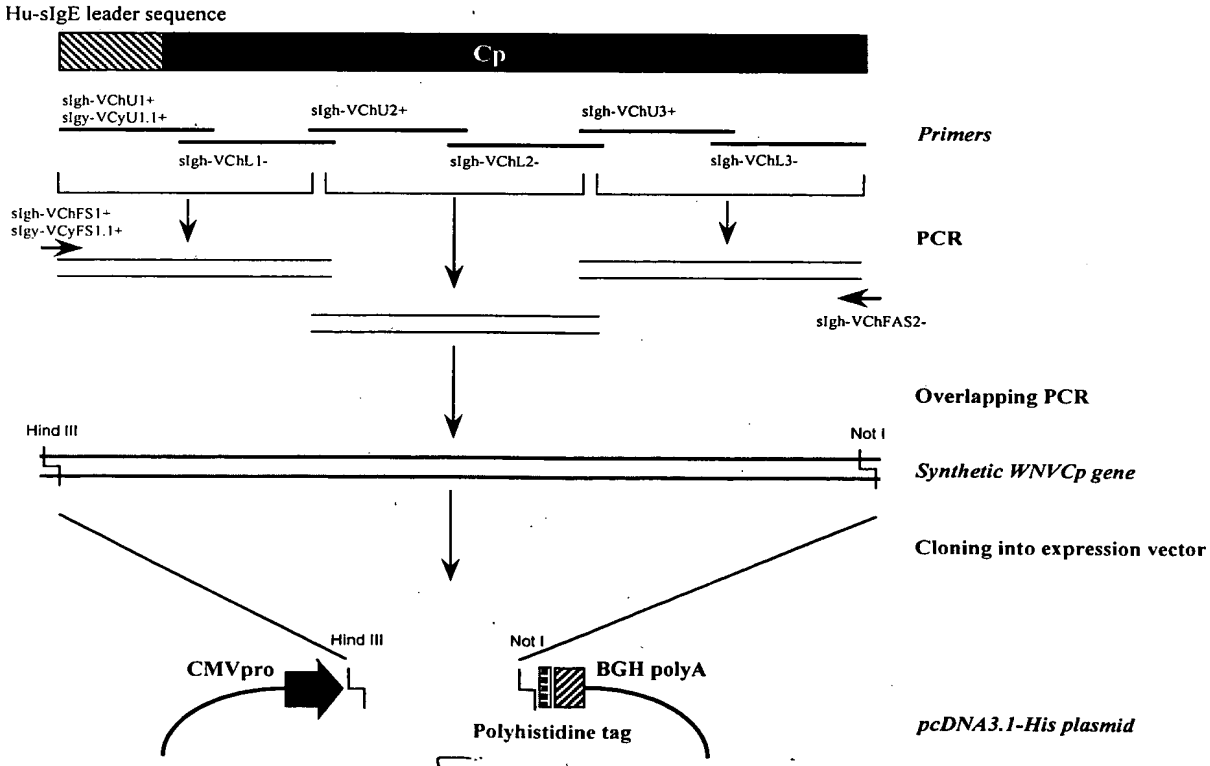


FIG 1

pWNVh-DJY Cut Site Map
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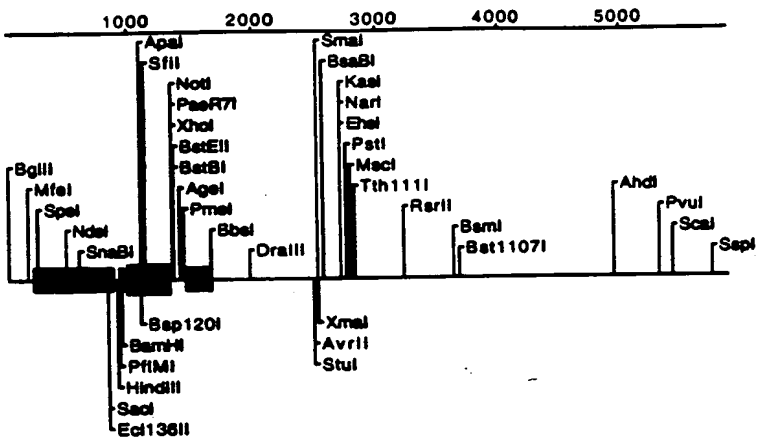

FIG. 2

pWNVh-DJY Cut Site Map
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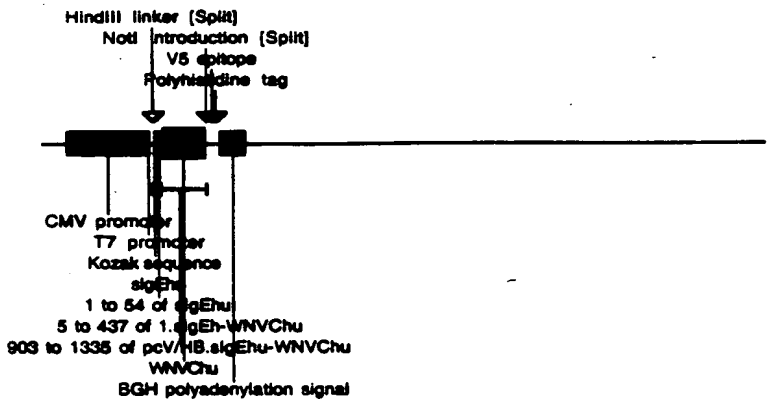
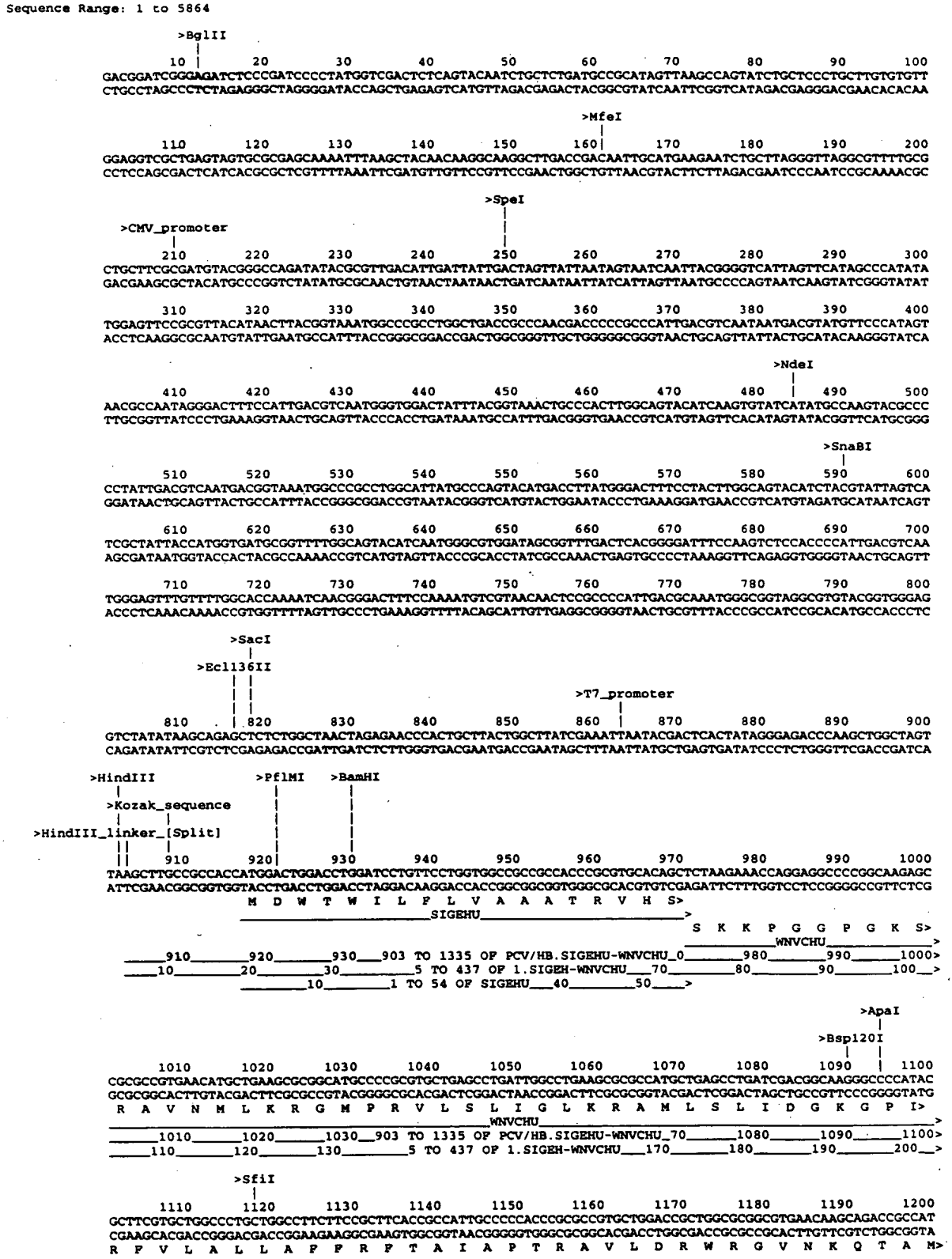


FIG. 3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SIGEHU
S K K P G G P G K S>
WNVCHU
910 920 930 903 TO 1335 OF PCV/HB.SIGEHU-WNVCHU_0 980 990 1000>
10 20 30 5 TO 437 OF 1.SIGEH-WNVCHU 70 80 90 100
10 1 TO 54 OF SIGEHU 40 50
>ApaI
>Bsp120I
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110 120 130 5 TO 437 OF 1.SIGEH-WNVCHU 170 180 190 200
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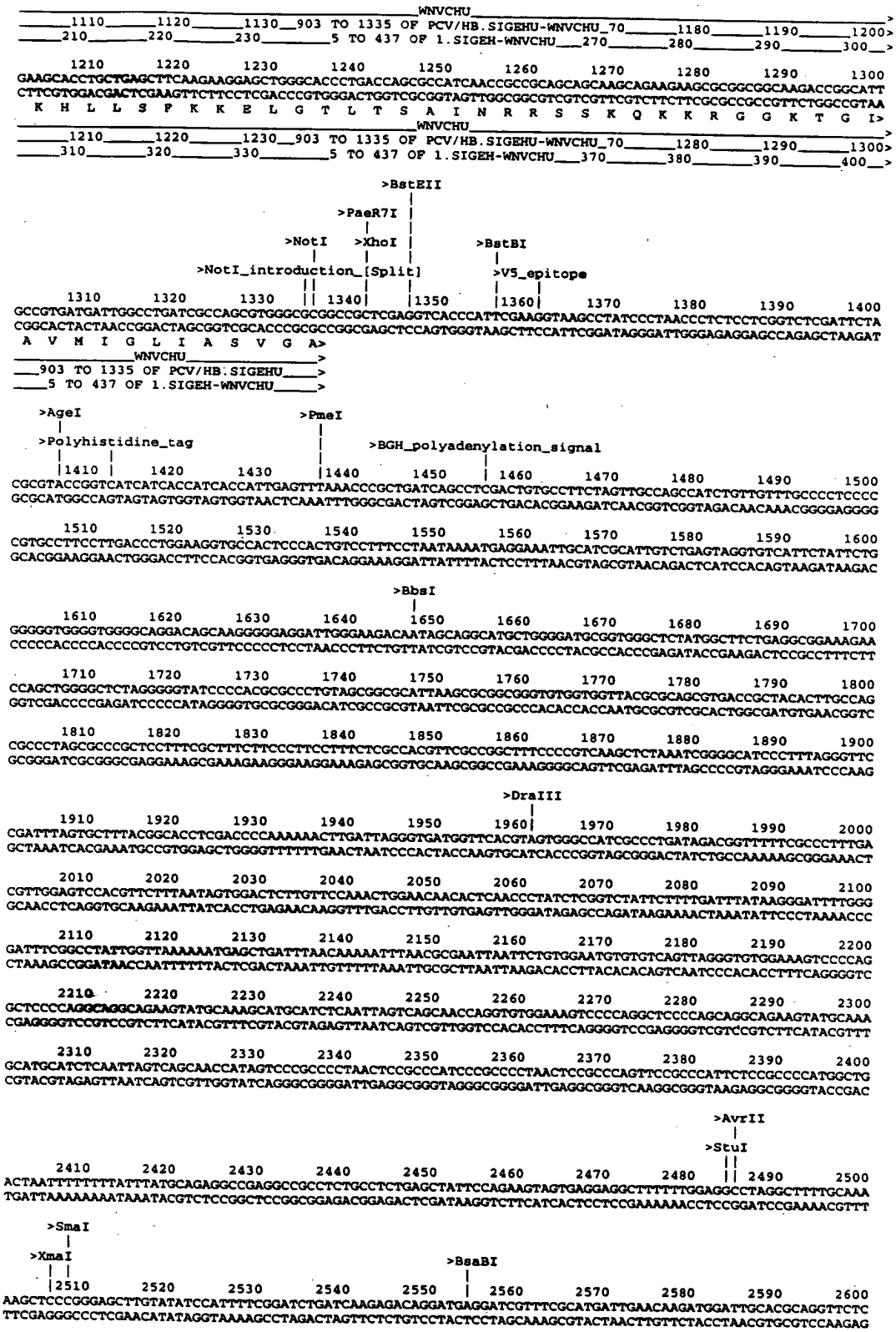
Fig 4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903 TO 1335 OF PCV/HB.SIGEHU-WNVCHU_70
5 TO 437 OF 1.SIGEH-WNVCHU
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>XhoI
>BstBI
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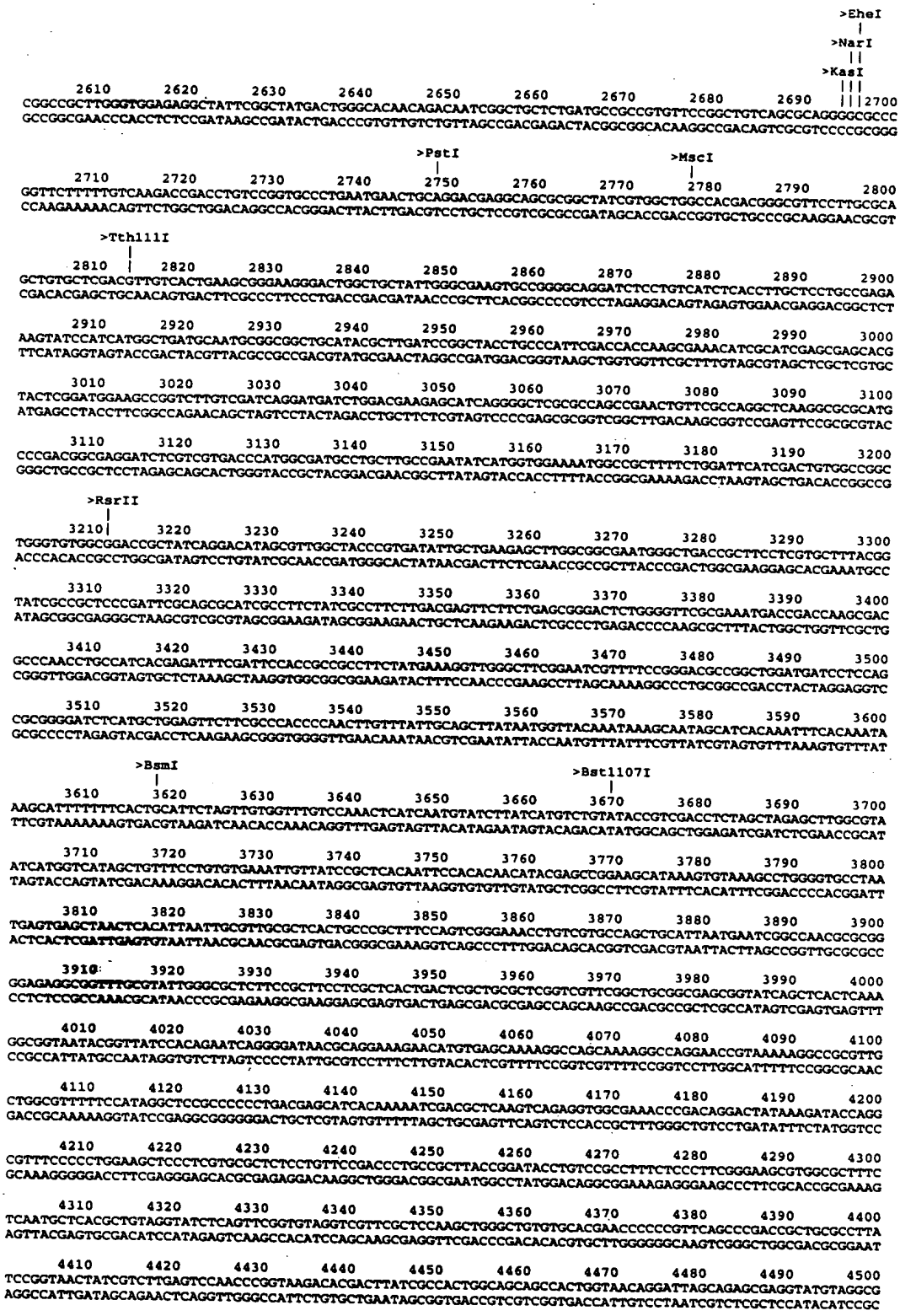
>XmaI
>BsaBI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                                          |
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      5210       5220       5230       5240       5250       5260       5270       5280       5290       5300
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          |
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                >ScaI
                  |
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      5510       5520       5530       5540       5550       5560       5570       5580       5590       5600
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                                          |
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      5810       5820       5830       5840       5850       5860
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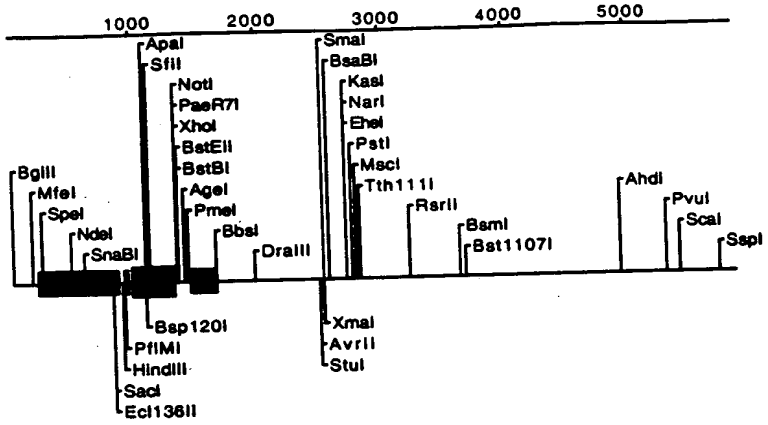
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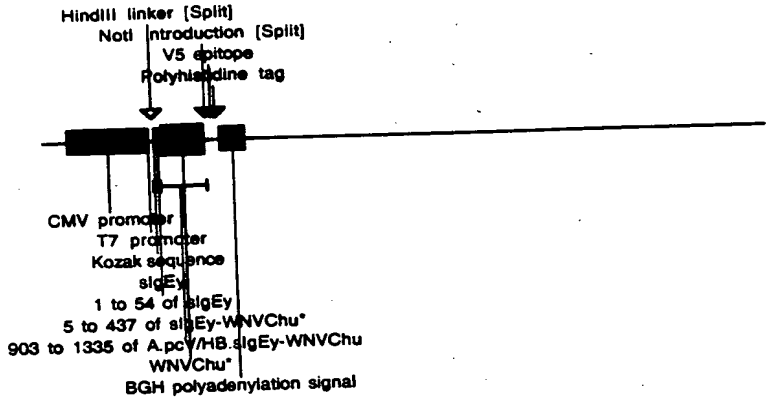
FIG. 5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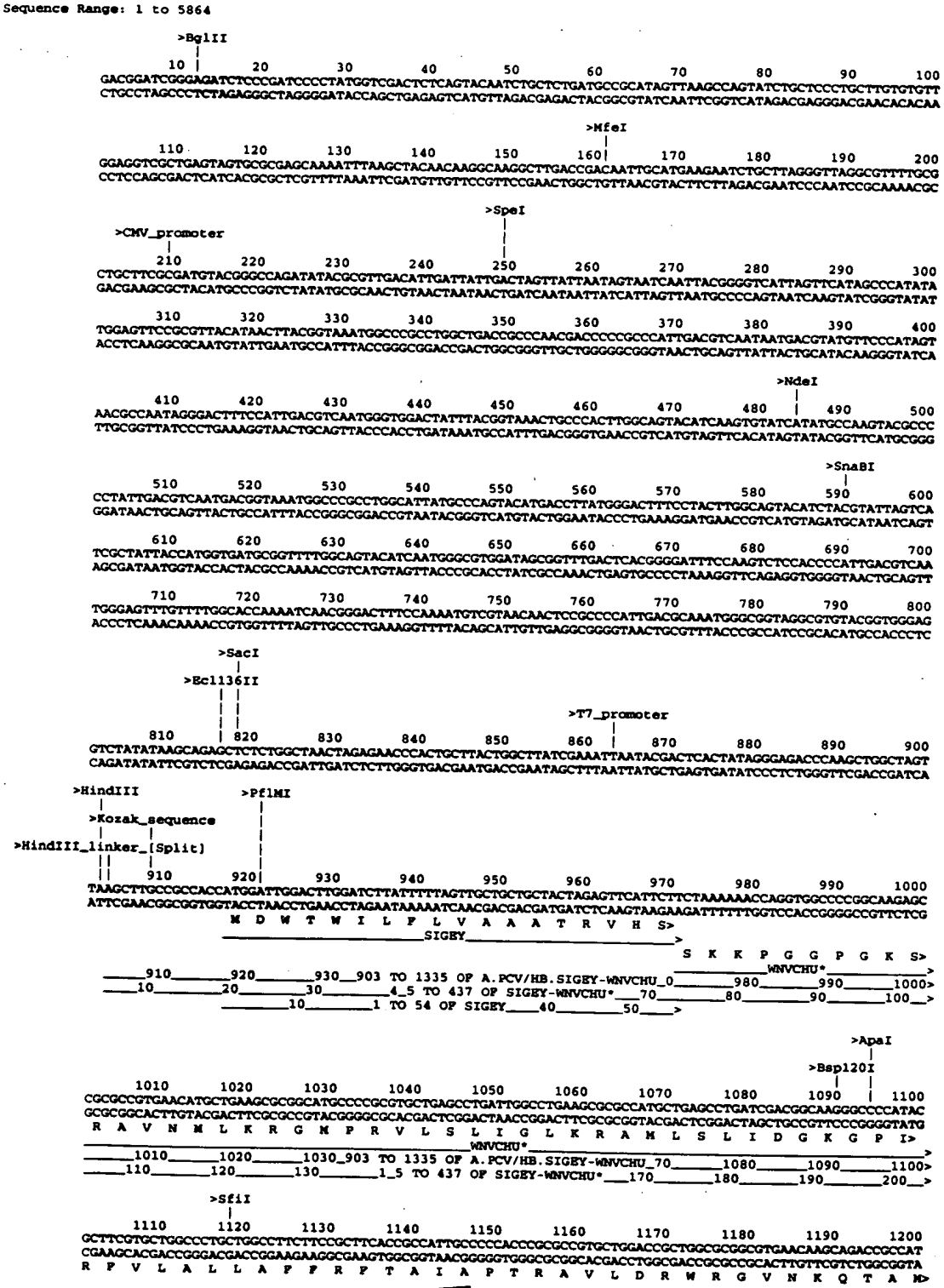
903 to 1335 of A.pcV/HB.sigEy-WNVChu
WNVChu*
BGH polyadenylation signal

FIG. 6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S K K P G G P G K S>
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910 TO 1335 OF A.PCV/HB.SIGEY-WNVCHU_0
4_5 TO 437 OF SIGEY-WNVCHU*
1 TO 54 OF SIGEY
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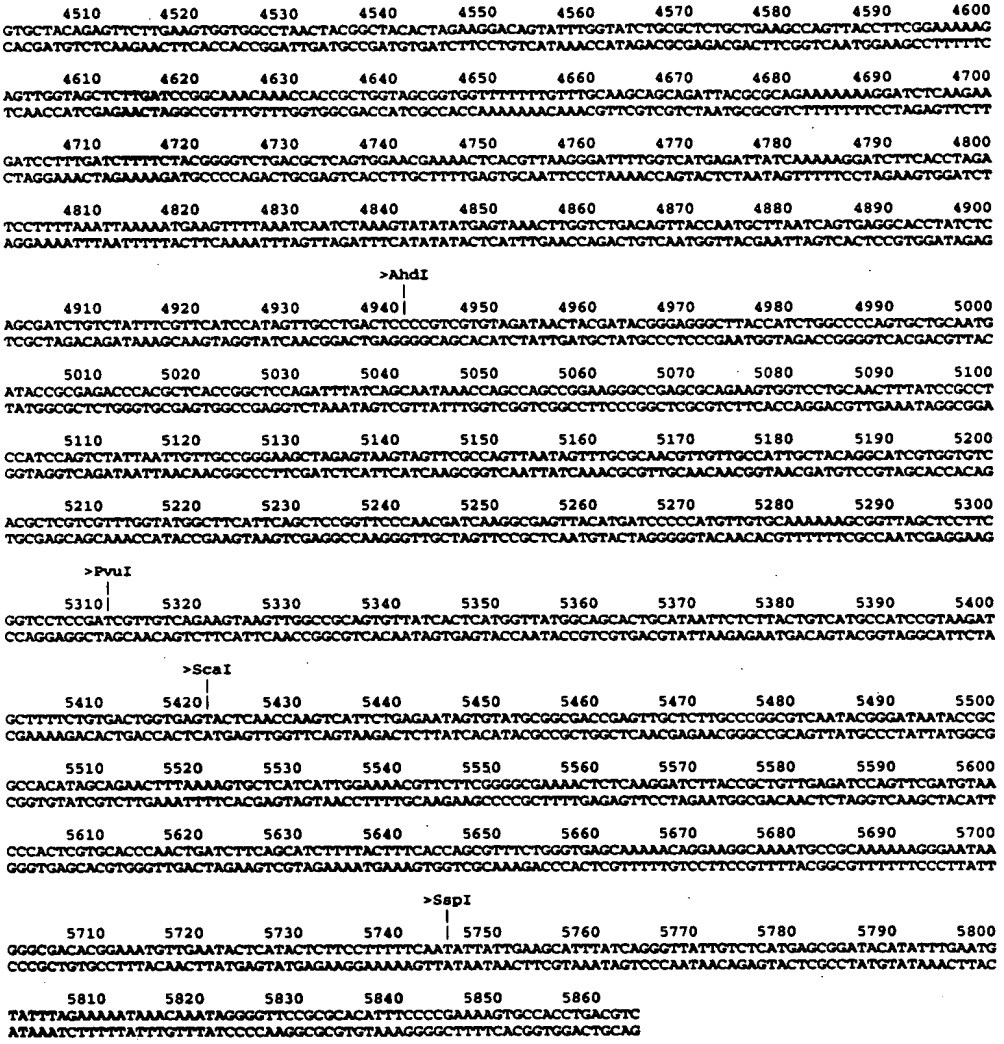
FIG 7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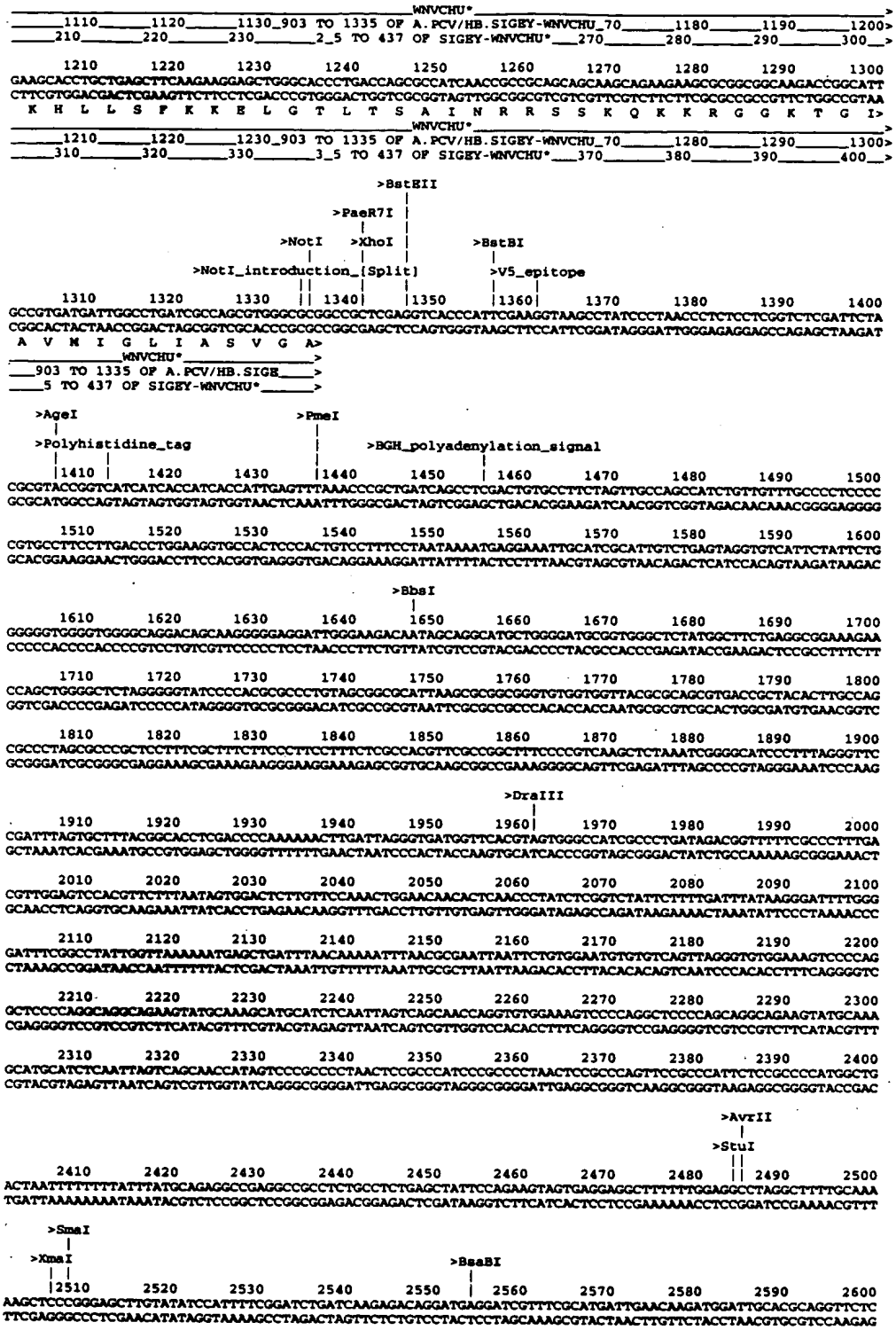
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>StuI
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TGATTAAAAAAAATAAATACGTCTCCGGCTCCGGCGGAGACGGAGACTCGATAAGGTCTTCATCACTCCTCCGAAAAAACCTCCGGATCCGAAAACGTTT
>SmaI
>XmaI
>BsaBI
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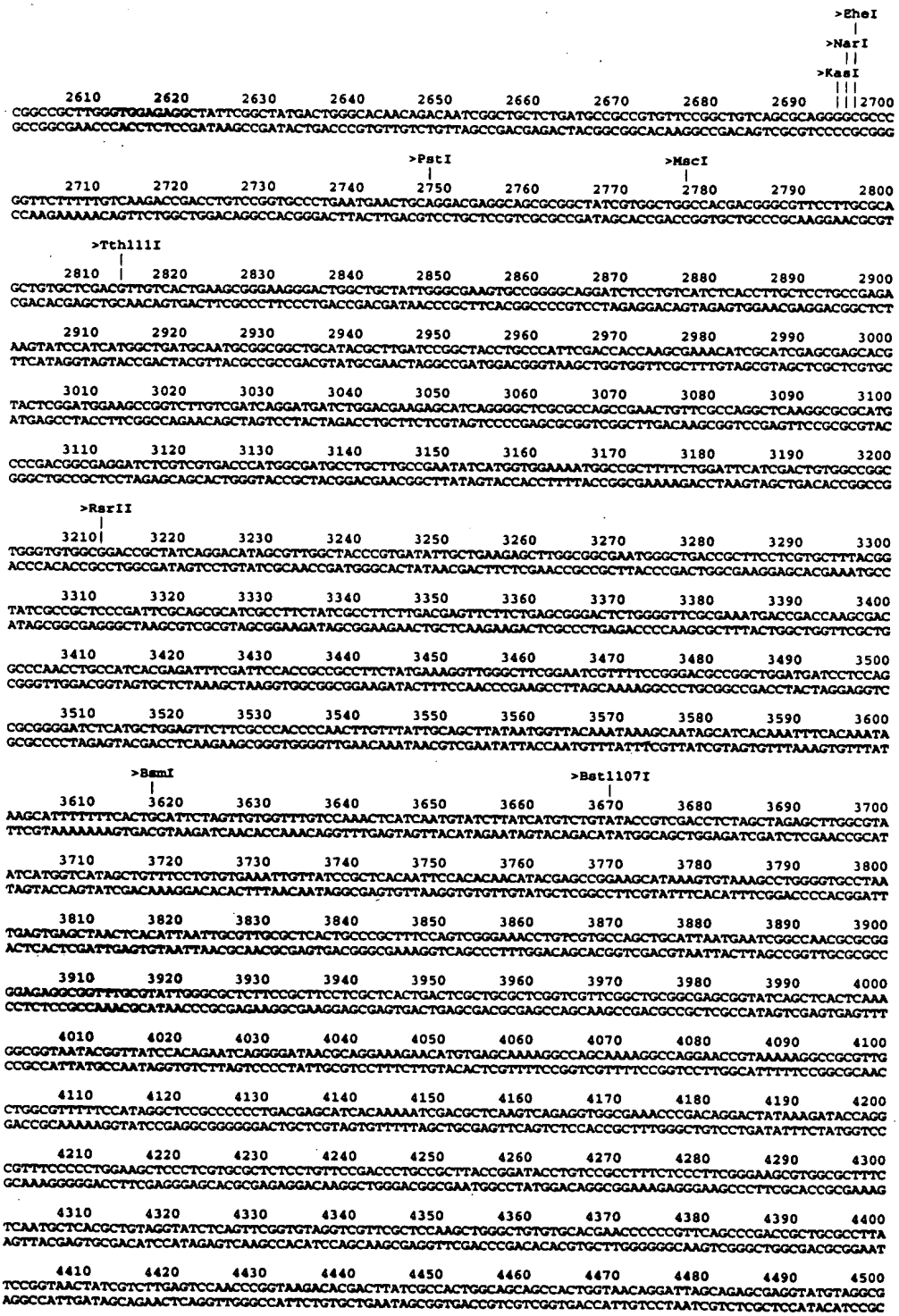
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>NarI
>KasI
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GCCGGCGAACCCACCTCTCCGATAAGCCGATACTGACCCGTGTTGTCTGTTAGCCGACGAGACTACGGCGGCACAAGGCCGACAGTCGCGTCCCCGCGGG
>PstI
>MscI
2710 2720 2730 2740 2750 2760 2770 2780 2790 2800
GGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCA
CCAAGAAAAACAGTTCTGGCTGGACAGGCCACGGGACTTACTTGACGTCCTGCTCCGTCGCGCCGATAGCACCGACCGGTGCTGCCCGCAAGGAACGCGT
>Tth111I
2810 2820 2830 2840 2850 2860 2870 2880 2890 2900
GCTGTGCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACCTTGCTCCTGCCGAGA
CGACACGAGCTGCAACAGTGACTTCGCCCTTCCCTGACCGACGATAACCCGCTTCACGGCCCCGTCCTAGAGGACAGTAGAGTGGAACGAGGACGGCTCT
2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
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TTCATAGGTAGTACCGACTACGTTACGCCGCCGACGTATGCGAACTAGGCCGATGGACGGGTAAGCTGGTGGTTCGCTTTGTAGCGTAGCTCGCTCGTGC
3010 3020 3030 3040 3050 3060 3070 3080 3090 3100
TACTCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCGCCAGGCTCAAGGCGCGCATG
ATGAGCCTACCTTCGGCCAGAACAGCTAGTCCTACTAGACCTGCTTCTCGTAGTCCCCGAGCGCGGTCGGCTTGACAAGCGGTCCGAGTTCCGCGCGTAC
3110 3120 3130 3140 3150 3160 3170 3180 3190 3200
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GGGCTGCCGCTCCTAGAGCAGCACTGGGTACCGCTACGGACGAACGGCTTATAGTACCACCTTTTACCGGCGAAAAGACCTAAGTAGCTGACACCGGCCG
>RsrII
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3310 3320 3330 3340 3350 3360 3370 3380 3390 3400
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ATAGCGGCGAGGGCTAAGCGTCGCGTAGCGGAAGATAGCGGAAGAACTGCTCAAGAAGACTCGCCCTGAGACCCCAAGCGCTTTACTGGCTGGTTCGCTG
3410 3420 3430 3440 3450 3460 3470 3480 3490 3500
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3510 3520 3530 3540 3550 3560 3570 3580 3590 3600
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>BsmI
>Bst1107I
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3710 3720 3730 3740 3750 3760 3770 3780 3790 3800
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TAGTACCAGTATCGACAAAGGACACACTTTAACAATAGGCGAGTGTTAAGGTGTGTTGTATGCTCGGCCTTCGTATTTCACATTTCGGACCCCACGGATT
3810 3820 3830 3840 3850 3860 3870 3880 3890 3900
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3910 3920 3930 3940 3950 3960 3970 3980 3990 4000
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CCTCTCCGCCAAACGCATAACCCGCGAGAAGGCGAAGGAGCGAGTGACTGAGCGACGCGAGCCAGCAAGCCGACGCCGCTCGCCATAGTCGAGTGAGTTT
4010 4020 4030 4040 4050 4060 4070 4080 4090 4100
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CCGCCATTATGCCAATAGGTGTCTTAGTCCCCTATTGCGTCCTTTCTTGTACACTCGTTTTCCGGTCGTTTTCCGGTCCTTGGCATTTTTCCGGCGCAAC
4110 4120 4130 4140 4150 4160 4170 4180 4190 4200
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GACCGCAAAAAGGTATCCGAGGCGGGGGGACTGCTCGTAGTGTTTTTAGCTGCGAGTTCAGTCTCCACCGCTTTGGGCTGTCCTGATATTTCTATGGTCC
4210 4220 4230 4240 4250 4260 4270 4280 4290 4300
CGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTC
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4310 4320 4330 4340 4350 4360 4370 4380 4390 4400
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4410 4420 4430 4440 4450 4460 4470 4480 4490 4500
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^{35}S-Labelled *in vitro* Translated Products of pWNVCh-DJY and pWNVCy-DJY

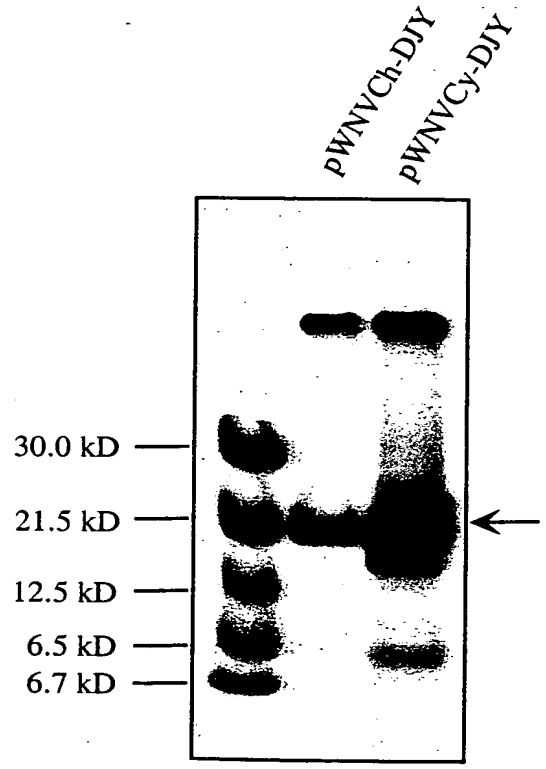


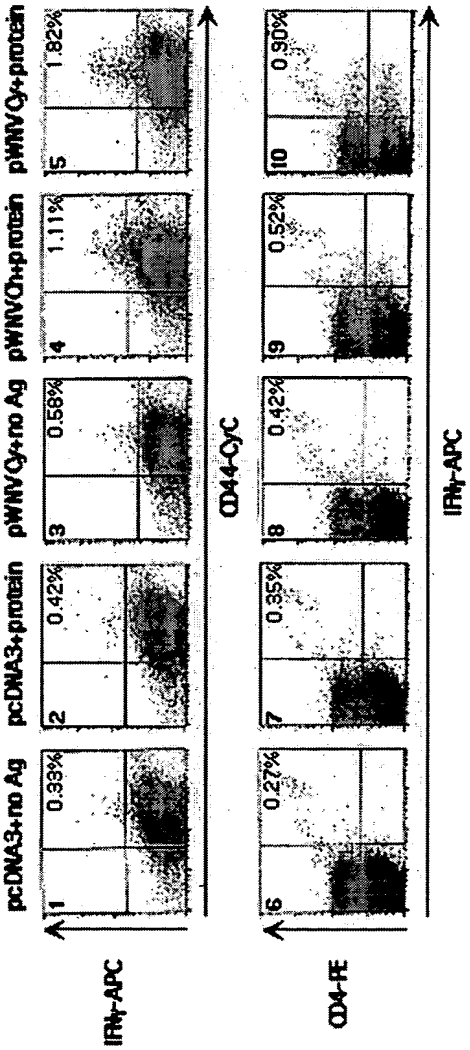
Fig 8

WNV Capsid (Cp) Peptides - Location and Sequences

WNV Cp	10	20	30	40	50	60	70	80	90	100	110	120
Amino Acid Sequence	MSKKPGGPGKSRAVNMLKRGMPRVLSLIGLKRAMLSLIDGKGPIRFVLALLAFFRFTAIAPTRAVLDRWRGVNKQTAMKHLLSFKKELGTLTSAINRRSSKQKKRGGKTGIAVMIGLIASVGA											
Peptide Seq.	SKKPGGPGKSRAVNMLKRGMPR			KRAMLSLIDGKGPIRFVLA						TLTSAINRRSSKQKKRGGKTGI		
Peptide Name	WNVC-P1			WNVC-P2						WNVC-P3		

Fig 9

Fig. 10


pcDNA3+no Ag
pcDNA3+protein
pWNVCy+no Ag
pWNVCh+protein
pWNVCy+protein
0.33%
0.42%
0.58%
1.11%
1.82%
0.27%
0.35%
0.42%
0.52%
0.90%
1
2
3
4
5
6
7
8
9
10
CD44-CyC
IFNγ-APC
CD4-PE
IFNγ-APC

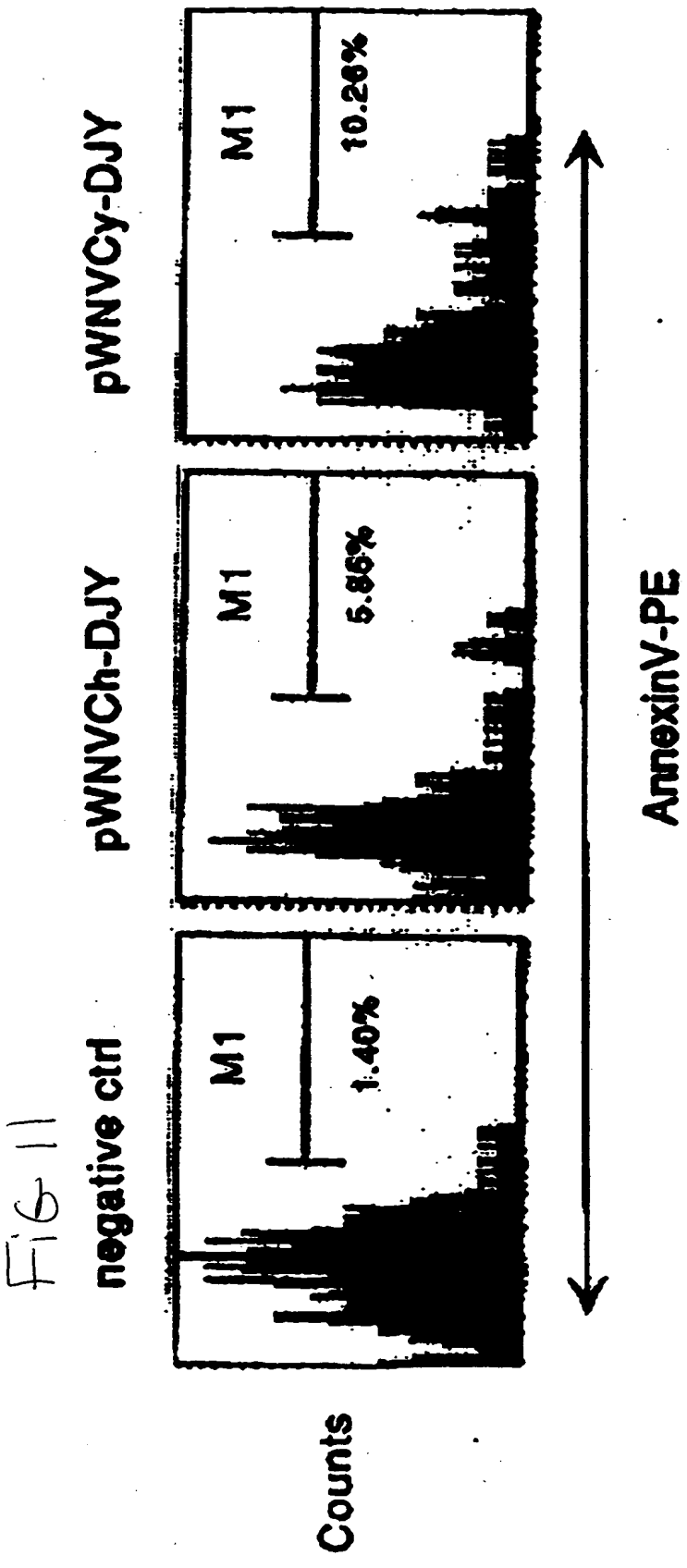

negative ctrl
M1
1.40%
pWNVCh-DJY
M1
5.86%
pWNVCy-DJY
M1
10.20%
Counts
AnnexinV-PE

Fig 11

Fig. 12A

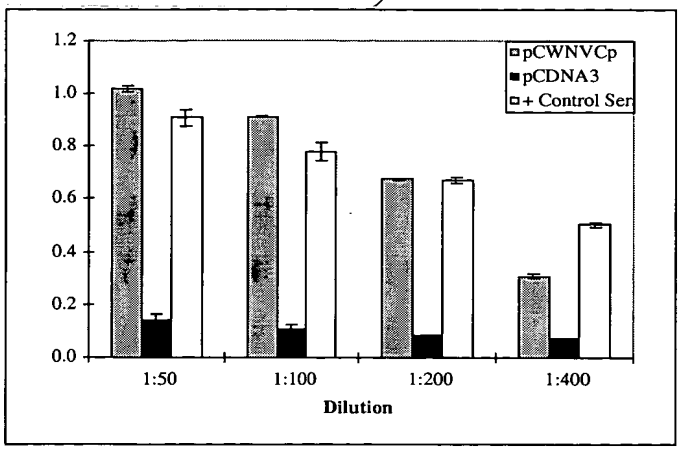


Fig. 12B

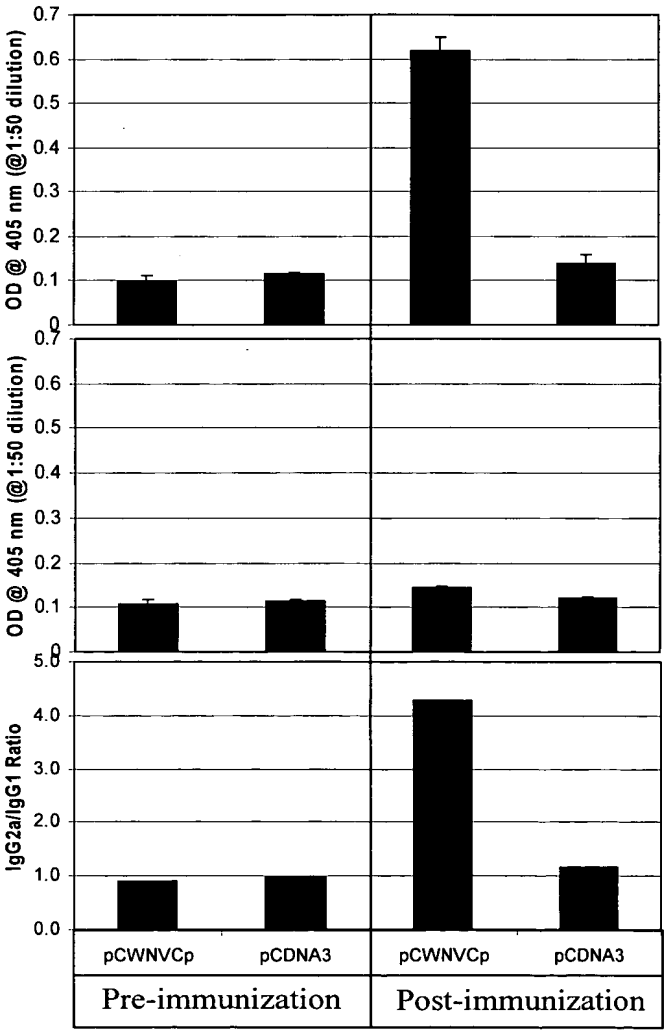
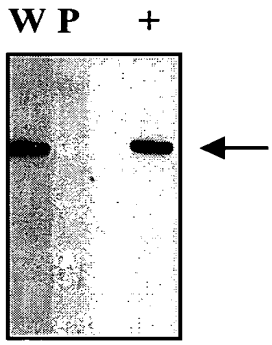


Fig. 12C



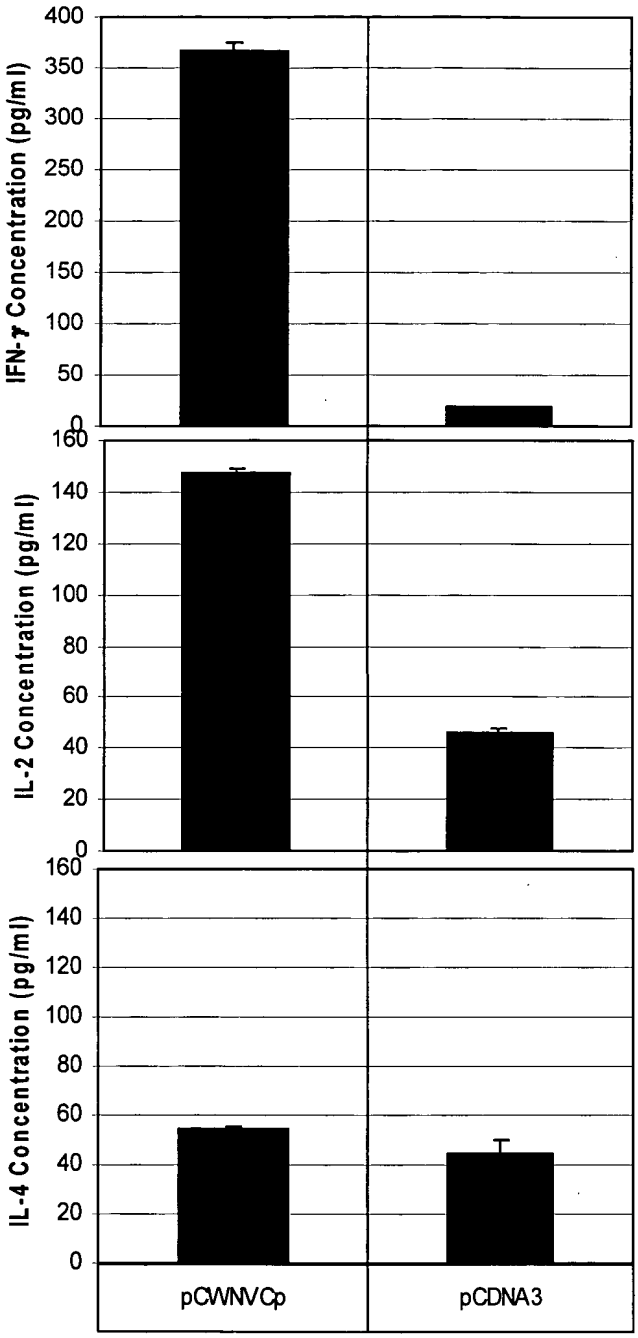

IFN-γ Concentration (pg/ml)
400
350
300
250
200
150
100
50
0
IL-2 Concentration (pg/ml)
160
140
120
100
80
60
40
20
0
IL-4 Concentration (pg/ml)
160
140
120
100
80
60
40
20
0
pCWNVCp
pCDNA3
Immunization Group

Figure 13

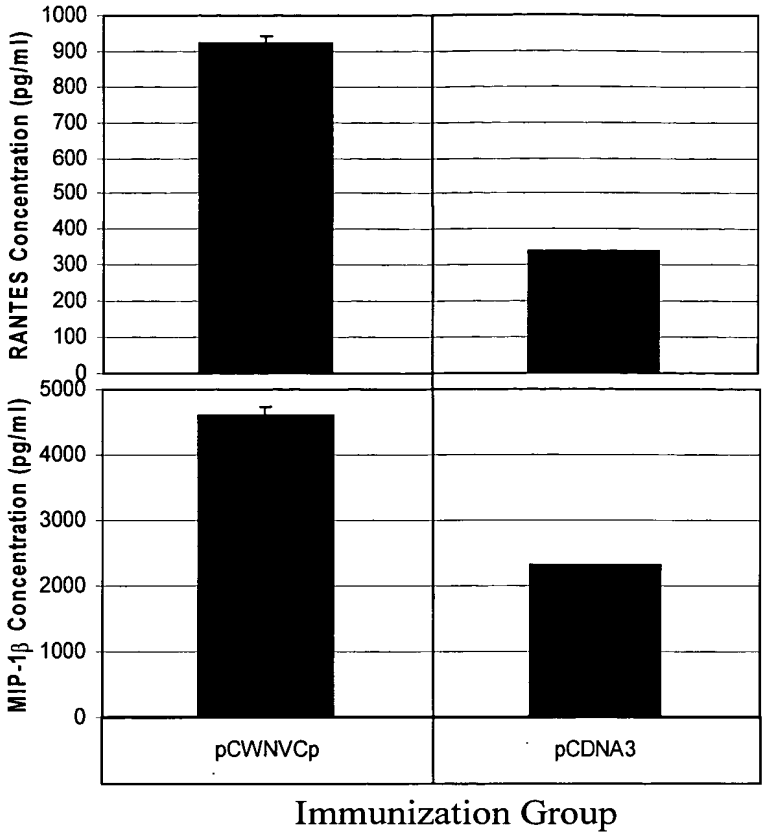

RANTES Concentration (pg/ml)
1000
900
800
700
600
500
400
300
200
100
0
MIP-1β Concentration (pg/ml)
5000
4000
3000
2000
1000
0
pCWNVCp
pCDNA3
Immunization Group

Figure 14

Fig. 15A

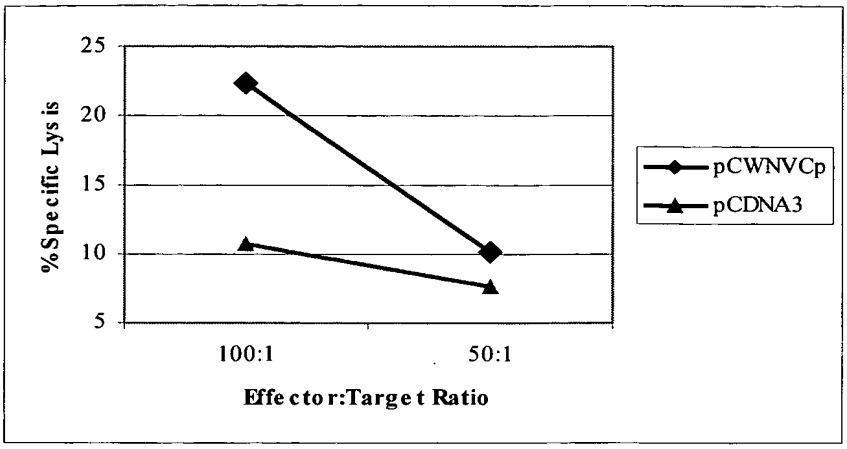


Fig. 15B

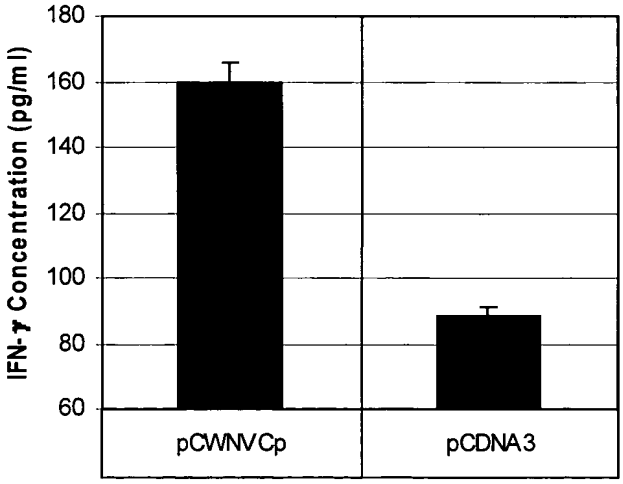


Fig. 16A

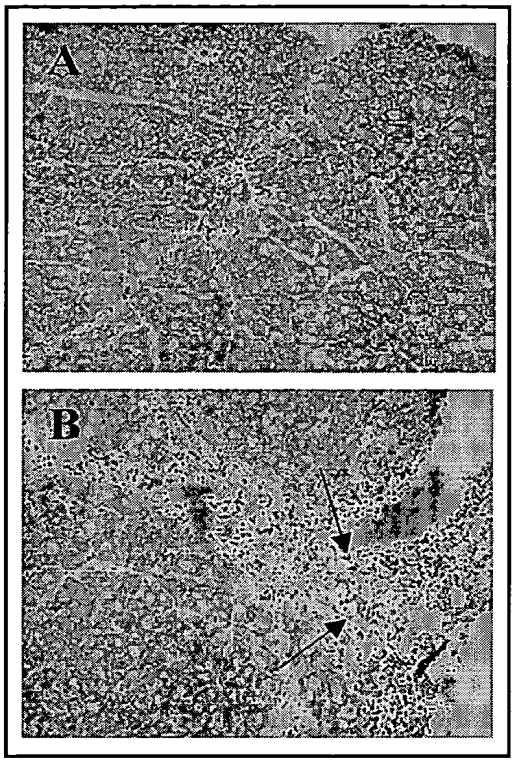


Fig. 16B

Fig. 16C

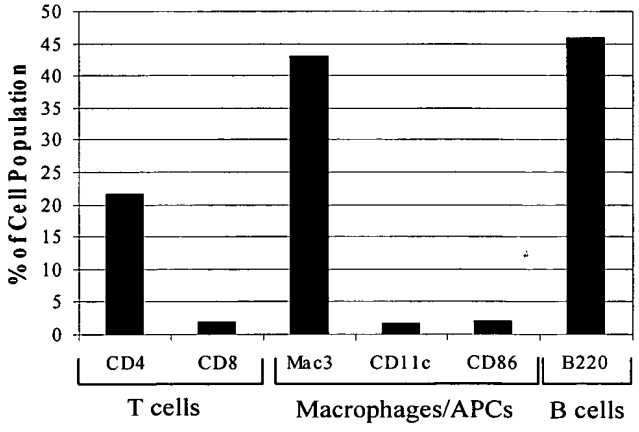


Figure 17

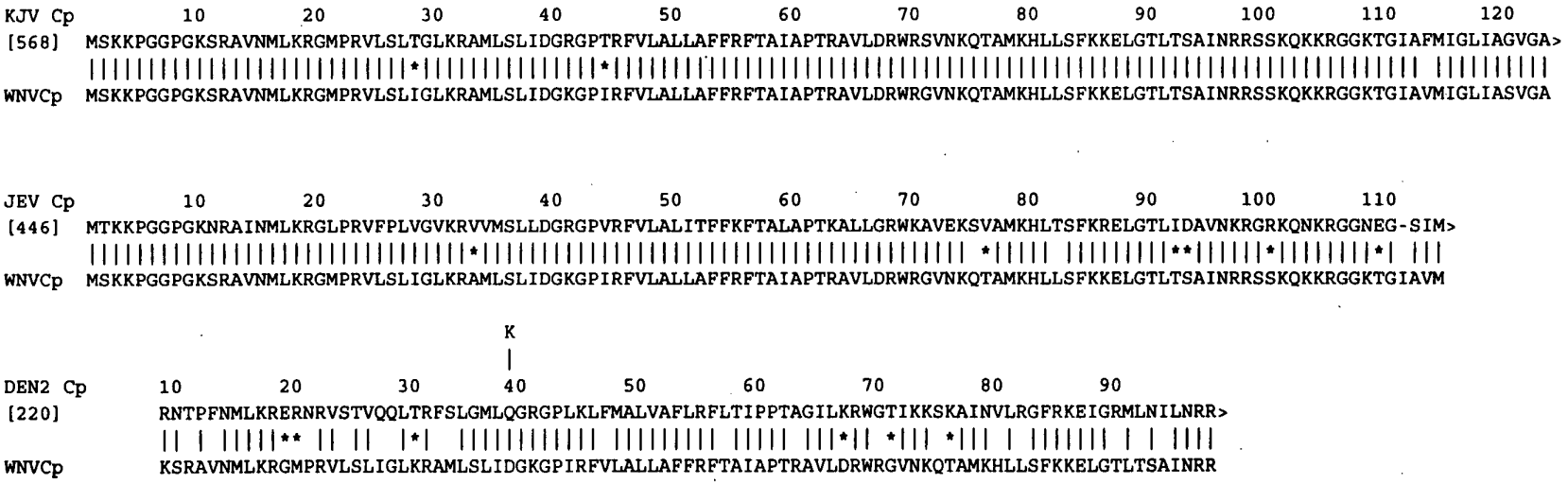


Fig. 18 Alignment of WNVCp protein sequence to other viral proteins

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Search from 1 to 123 where origin = 1      Score Region from 1 to 123
Date: June 15,2001                         Maximum possible score: 590
Time: 19:48:02

Database: UserFolder: Alignment-AC6/01

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WNVCaa   MSKKPGGPGKSRAVNMLKRGMPRVLSLIGLKRAMLSLIDGKGPIRFVLALLAFFRFTAIAPTRAVLDRWRGVNKQTAMKHLLSFKKELGTLTSAINRRSSKQKKRGGKTGIAVMIGLIASVGA

1. HIV-1 89.6 Vpr protein
1. HIV-1 89.                                60         70         80         90
[   41 ]                                    TWTGVEALIRILQQLLFI-HFR-IGCRHSRIGIIQQRRT-RNG>

WNVCaa                                      RWRGVNKQTAMKHLLSFKKELGTLTSAINRRSSKQKKRGGKTG

2. Herpes Simplex virus major capsid protein
                                                                                                              VR
                                                                                                              |
2. HerpesMaj   160       170       180       190       200       210       220       230       240       | 250       260
[   64 ]   AVKTV-ASALQFGVD-ALER-GLINTVLSVKLRHAPPMFILQTLADPTFTERGFSKTVKSDLIAMFKRHLLEHSFF-LDRAENMGSGFSQYSRLSEMVAAVSGESVLKGV>

WNVCaa     PGKSRAVNMLKRGMPRVLSLIGLKRAMLSLIDGKGPIRFVLALLAFFRFTAIAPTRAVLDRWRGVNKQTAMKHLLSFKKELGTLTSAINRRSSKQKKRGGKTGIAVMIGL

3. Ebola nuclear protein
                                                   S
                                                   |
3. EBOLANucl    200       210       220       230  | 240       250       260       270       280       290       300
[   48 ]     HMMVIFRLMRTN-FLIKFLL-IHQGMHMVAGHDANDAVISNVAQARFSGLLIVKTVLDHILQKTERGVRLHPLARTAKVKNEVNSFKAALSSLAKHGEYAPFARLLNL>

WNVCaa       KSRAVNMLKRGMPRVLSLIGLKRAMLSLIDGKGPIRFVLALLAFFRFTAIAPTRAVLDRWRGVNKQTAMKHLLSFKKELGTLTSAINRRSSKQKKRGGKTGIAVMIGL
```

4. Ebola glycoprotein

```
4. EbolaGlyc       120       130
[   30 ]       KPDGSECLPAAPDGIRGFPR>
               |||||     |     || ||
WNVCaa         KPGGPGKSRAVNMLKRGMPR
```

5. Ebola glycoprotein

```
5. EbolaGlyc
[   39 ]

WNVCaa
```

6. Rubella capsid protein

```
6. Rubellaca
[   38 ]

WNVCaa
```

```
570       580       590
LQLFLRATTELRTFSILNRK-AID>
|  ||| ||   | |||||  |||
LLAFFRFTAIAPTRAVLDRWRGVN
```

```
270         280         290         300         310
RSARHPWRI-R-FGAPQAFLAG-LLLATVAVGTAR-AGLQP-RADMAAPPTL>
||  | ||      | | |||    ||||| |  | || |  ||| || | |
RAVLDRWRGVNKQTAMKHLLSFKKELGTLTSAINRRSSKQKKRGGKTGIAVM
```

Docket Number: UPN-4105 (M2351)
Title: Compositions and Methods of Using Caps
Protein from Flaviviruses and Pestiviruses
Inventors: David B. Weiner and Joo-Sung Yang
Sheet 25 of 30

Fig. 18(continued) Alignment of WNVCp protein sequence to proapoptotic proteins

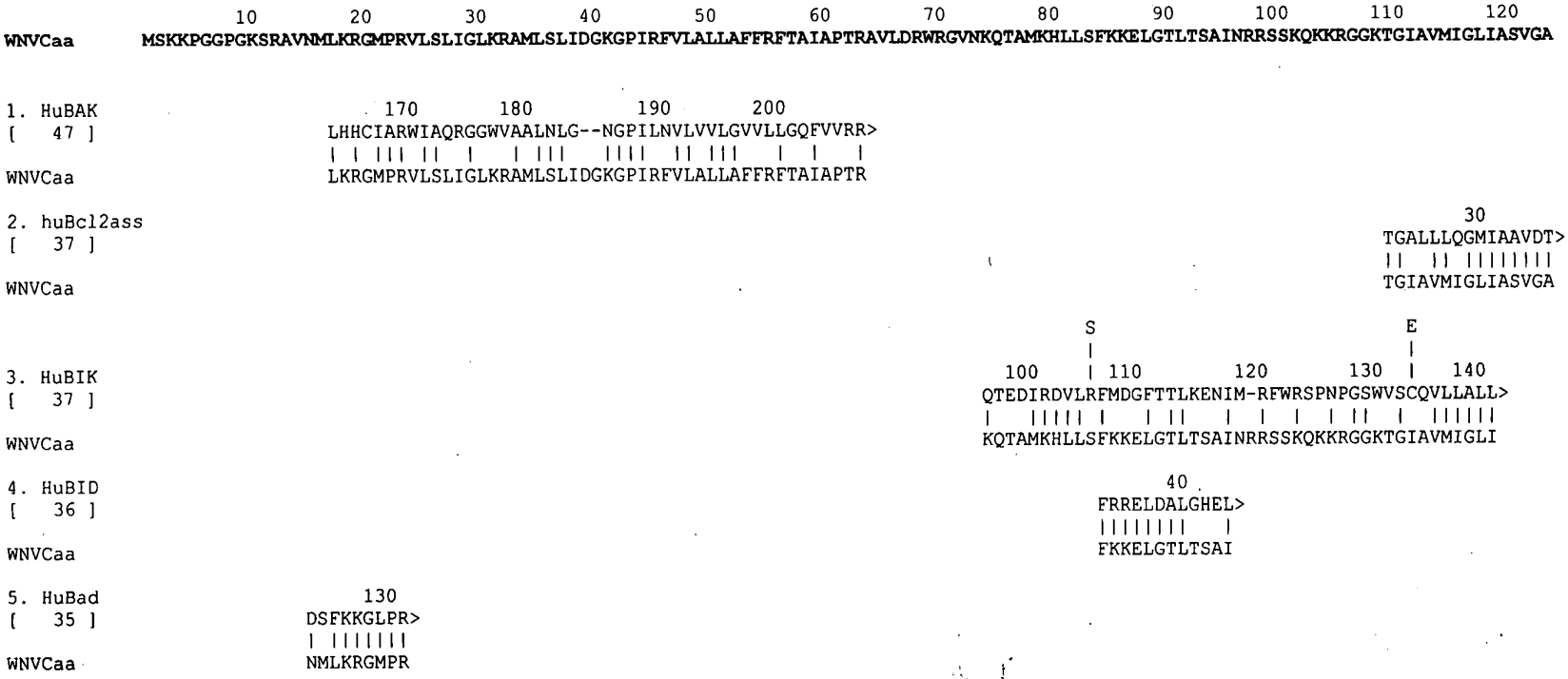

Fig. 19 Alignment of HIV-1 89.6 Vpr protein sequence to other viral proteins

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Search from 1 to 96 where origin = 1 Score Region from 1 to 96
Date: June 15,2001 Maximum possible score: 515
Time: 19:57:09

Database: UserFolder: Alignment-AC6/01

```
                  10        20        30        40        50        60        70        80        90
HIV-1 89.6  MEQAPEDQGPQREPYNDWTLELLEELKNEAVRHFPRIWLHSLGQHIYETYGDTWTGVEALIRILQQLLFIHFRIGCRHSRIGIIQQRRTRNGASKS
```

1. p230 nonstructural protein/ Sindbis virus

```
1. p230nonst                        1320      1330      1340
[   50 ]                          FRQLDNSRTRQFTPHHLNCVISSVYEG--T-RDGVGA>
                                  | || |   |||    || |   |||     | || |
HIV-1 89.6                        LEELKNEAVRHFPRIWLHSLGQHIYETYGDTWTGVEA
```

2. West Nile Virus capsid protein

```
2. WNVCaa                                                  70        80        90       100       110
[   45 ]                                                 RWRGVNKQTA-MKHLLSFKKELGTLTSAINRRSSKQKKRGGKTG>
                                                          | |||     ||||| |   ||   | |     || | ||  |
HIV-1 89.6                                               TWTGVEALIRILQQLLFIHFRIGCRHSRIGIIQQRRTRNGASKS
```

3. Cucumovirus 2A protein

```
3. Cumbermos                                                10
[   36 ]                                                  DFHRFSYIRDRRA>
                                                            ||| ||||||
HIV-1 89.6                                                RHSRIGIIQQRRT
```

4. Cucumovirus 2A protein

```
4. Cumbermos                                        110       120
[   40 ]                                             EFGNTFSVPDPLR-EVQRL>
                                                      |||| |  |||   ||||
HIV-1 89.6                                           TYGDTWTGVEALIRILQQL
```

5. Rubella virus capsid protein

```
5. Rubellaca                             160
[   33 ]                                WLWSEGQGAVFY>
                                        ||| || |  ||
HIV-1 89.6                              RIWLHSLGQHIY
```

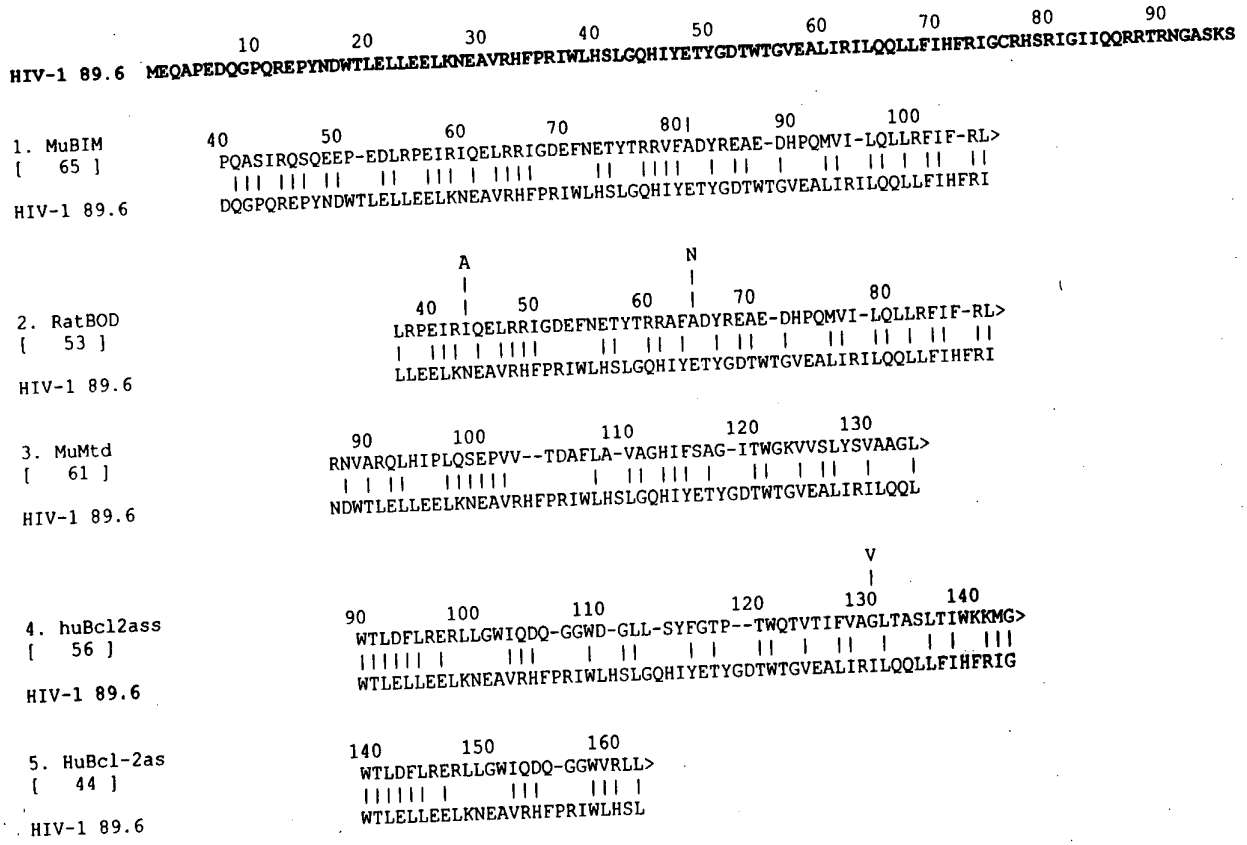
6. Nipah virus fusion protein

```
6. NipahFusi
[   44 ]                                                       .sky.sd...vf->
                                                               | | | |||||
HIV-1 89.6                                                     LIRILQQLLFIHF
```

7. Reovirus core-minor form Mu2

```
7. Minor-Reo     410        420        430
[   46 ]          IGAVLPKGSFKSTIMRVLDEMEVLGVRIMPR>
                   |    | |||   | |||||   |||  ||
HIV-1 89.6        EDQGPQREPYNDWTLELLEELKNEAVRHFPR
```

Fig. 19(continued) Alignment of HIV-1 89.6 Vpr protein sequence to proapoptotic proteins



6. HuBad
[36]

HIV-1 89.6

150 160
VFQSWWDRNLGR>
| | |||||
HFPRIWLHSLGQ